**ORIGINAL ARTICLE** Iran J Allergy Asthma Immunol December 2011; 10(4): 237-242

## In vitro Soluble CD30 Levels in Patients with Chronic Stable Coronary Artery Disease

Mohammad Jafar Mahmoudi<sup>1</sup>, Mona Hedayat<sup>1,2</sup>, Nima Rezaei<sup>2,3</sup>, Ali-Akbar Saboor-Yaraghi<sup>4</sup>, and Maryam Mahmoudi<sup>4</sup>

 <sup>1</sup> Division of Cardiology, Department of Internal Medicine, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
<sup>2</sup> Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
<sup>3</sup> Molecular Immunology Research Center and Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
<sup>4</sup> Department of Nutrition and Biochemistry, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Received: 15 February 2011 ; Received in revised form: 2 July 2011 ; Accepted: 17 July 2011

# ABSTRACT

The CD30 antigen seems to play a costimulatory role in maintaining the physiological balance between T-helper (Th)1/Th2 immune responses. In this study, plasma and *in vitro* soluble CD30 (sCD30) secretion was investigated in patients with coronary artery disease (CAD) as a plausible marker of dysregulated immune response.

Twenty one patients with angiographically confirmed CAD and 31 healthy controls took part in this study. The levels of the activation marker sCD30 were determined in plasma and phytohaemagglutinin (PHA)-stimulated and unstimulated peripheral blood mononuclear cell cultures by ELISA.

Plasma sCD30 levels did not differ significantly between the patients and controls. However, spontaneous sCD30 secretion was significantly lower in patients with CAD compared to controls ( $p \le 0.001$ ). The soluble CD30 levels were significantly increased in the supernatant of PHA-stimulated PBMCs compared to unstimulated cultures in both groups of patients and controls ( $p \le 0.001$ ). PHA-stimulated sCD30 secretion was found to be lower in patients compared to controls; however, the difference was not statistically significant.

Plasma sCD30 levels were not statistically different in patients with chronic stable CAD, a well-known Th1-mediated disease, compared to controls; whereas decreased spontaneous and PHA-stimulated sCD30 secretion in patients with CAD might indicate the progressive shift towards a Th1 immune response.

Keywords: Coronary artery disease; Soluble CD30; T-helper

**Corresponding Author:** Maryam Mahmoudi, MD; PhD; Department of Nutrition and Biochemistry, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. Tel: (+9821) 8897 4463, Fax: (+98 21) 8897 4462, E-mail: mahmoodi\_maryam@yahoo.com

#### INTRODUCTION

Atherosclerosis is a chronic inflammatory disease of the arterial wall characterized by progressive lipid

Copyright© 2011, IRANIAN JOURNAL OF ALLERGY, ASTHMA AND IMMUNOLOGY. All rights reserved.

accumulation, inflammatory cell infiltrates, cell death, and fibrosis.<sup>1</sup> The immune system, encompassing both innate and adaptive immunity, has been implicated in the atherogenic process.<sup>2,3</sup> Despite the fact that the precise role of various T-cell subsets in atherogenesis is far from clear, the proatherogenic role of T-helper type 1 (Th1) cells is well established, promoting inflammation and matrix degradation.<sup>4</sup>

CD30 is a membrane glycoprotein of the tumor factor receptor (TNFR) superfamily,<sup>5</sup> necrosis originally identified as a cell surface antigen on Reed-Sternberg cells of Hodgkin lymphoma.<sup>6</sup> In normal conditions, CD30 expression depends on cell activation and proliferation and is largely restricted to activated T, B, and natural killer cells.<sup>7</sup> Previous studies have vielded conflicting results concerning the preferential expression of CD30 on lymphocytes expressing a Th2 phenotype.<sup>8-11</sup> One explanation would be that CD30 has been found to be transiently expressed on normal activated T cells, whereas its expression persists on T cells of Th2 profile.<sup>7,12</sup> Far more than being a mere marker, CD30 exerts pleiotropic biological functions,<sup>8,12</sup> which, of note, is its costimulatory role in maintaining the physiological balance between Th1/Th2 immune responses.<sup>13-15</sup>

A soluble form of CD30 (sCD30) has been found to be closely related to cell CD30 expression.<sup>16,17</sup> Increased sCD30 levels have been reported in a variety of immunopathological disorders;<sup>12,18,19</sup> however, it is still a matter of debate whether abnormal increases or decreases in sCD30 levels are associated with impaired regulation.<sup>14,15</sup> Indeed, to our best of knowledge, this is the first time that this biomarker is assessed in coronary artery disease (CAD). The purpose of the present study was to investigate plasma and *in vitro* secretion of sCD30 in phytohaemagglutinin (PHA)-stimulated and unstimulated peripheral blood mononuclear cell (PBMC) cultures in patients with chronic stable CAD, as a plausible marker of dysregulated immune response, in comparison with healthy controls.

### MATERIALS AND METHODS

#### **Participants**

In the present study, a total of 52 participants who underwent elective coronary angiography at the Catheterization Laboratory of Imam Khomeini Hospital Complex, Tehran University of Medical Sciences for the evaluation of stable CAD based on clinical indication were enrolled. Twenty one patients with significant CAD, defined as  $\geq$  50% diameter stenosis in at least one of the major coronary arteries, and 31 subjects with no CAD took part in this study. Subjects with active infections, autoimmune diseases, malignancies, and recent myocardial infarction were excluded. Written informed consent was obtained from all participants prior to blood sampling. This study was approved by the Ethics Committee of Tehran University of Medical Sciences and Health Services.

#### **Cell Culture**

The heparinized blood samples were centrifuged and the plasma was collected and stored at -80°C until assayed. PBMCs were isolated by the Ficoll-Hypaque gradient centrifugation method. The remaining whole blood was diluted 1:2 with sterile phosphate-buffered saline PH=7.4 and layered onto half the volume of Ficoll-Histoprep (BAG Health Care GmbH, Germany). The sample was centrifuged and the interface layer containing the mononuclear cells was collected. Cell viability and cell counts were assessed by Trypan blue exclusion method. Mononuclear cells were resuspended in culture medium (RPMI-1640 supplemented with 10% heat inactivated fetal calf serum; Gibco, Invitrogen, UK), diluted to  $1 \times 10^6$  cells per mL, and plated at a density of  $1.5 \times 10^5$  cells per well in 96-well flat-bottomed microtiter plates. Cells were cultured for 66 hours in the presence of 10 µg/mL PHA (Sigma, USA) or medium alone. Culture supernatants were harvested and frozen at -80°C for sCD30 assay.

#### sCD30 Assay

sCD30 concentrations were measured in plasma and supernatants of PBMC using commercially available Enzyme-Linked Immunosorbent Assay (ELISA; Bender MedSystems, Vienna, Austria). The optical density was determined at 450 nm using micro ELISA plate reader. sCD30 concentration was read from the standard curve generated using recombinant human sCD30 provided with the assay kit. The results obtained were expressed as ng/mL.

#### **Statistical Analyses**

The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Parametric and nonparametric variables were presented as mean  $\pm$  SD and median (range), respectively. Mann-Whitney U test and

Wilcoxon signed ranks test were used to compare variables between groups. A p value < 0.05 was considered statistically significant.

#### RESULTS

Twenty one patients with angiographically confirmed CAD aged 40-69 (51.62  $\pm$  9.13) years and 31 healthy controls with no coronary artery involvement aged 42-66 (49.90  $\pm$  7.04) years took part in this study (p = 0.161). Nineteen out of 21 (90.47%) patients and 28 out of 31 (90.32%) controls were male.

Plasma sCD30 levels did not differ significantly between the patients and controls (p = 0.225); whereas, *in vitro* spontaneous sCD30 secretion was significantly lower in patients with CAD (median: 0.00 ng/mL, range: 0.00-2.01 ng/mL) compared to controls (median: 0.00 ng/mL, range: 0.00-8.90 ng/mL) (p < 0.001) (Figure 1).

Following PHA stimulation, the levels of sCD30 secretion were significantly increased in both groups of patients (p < 0.001) and controls (p < 0.001). Likewise, PHA-stimulated sCD30 secretion was found to be lower in patients with CAD (median: 7.59 ng/mL, range: 0.00-22.60 ng/mL) in comparison with controls (median: 8.64 ng/mL, range: 0.00-34.04 ng/mL), but this difference was not statistically significant (p = 0.332).

#### DISCUSSION

CD30 has been found to be an important costimulatory molecule for maintaining the physiological balance between Th1/Th2 immune responses.<sup>13-15</sup> Despite early reports of normal serum sCD30 levels in Th1-driven diseases,<sup>20,21</sup> increased levels of sCD30 and/or CD30 expression have been demonstrated in subsequent studies of Th1-polarized immune responses, including tuberculosis,<sup>22</sup> Wegner's granulomatosis,<sup>23</sup> Hashimoto's thyroiditis,<sup>24</sup> Graves' disease,<sup>24</sup> Graves' ophthalmopathy,<sup>25</sup> primary biliary cirrhosis,<sup>26</sup> Sjogren's syndrome,<sup>27,28</sup> and rheumatoid arthritis.<sup>29,30</sup>

It has been postulated that CD30<sup>+</sup> T cells play a counter-regulatory role in rheumatoid arthritis and, by extension, in other Th1-mediated conditions, with increased circulating sCD30 levels reflecting such cell activity.<sup>31</sup>

This may have been partly due to increased production of interleukin (IL)-4 and IL-10 by CD30<sup>+</sup> T cells.<sup>30</sup> Indeed, the production of Th2-type cytokines counteracts the deleterious effects of proinflammatory Th1-type cytokines in an attempt to resolve the disease process.<sup>32-34</sup>



Figure 1. Spontanous and PHA-stimulated sCD30 secretion (ng/mL) in patients with CAD (n=21) and controls (n=31). Boxes represent values between the  $25^{th}$  and  $75^{th}$  percentiles. The horizontal lines correspond to the median, minimum and maximum. A *p* value < 0.05 was considered statistically significant.

The serum sCD30 levels were inversely correlated with the inflammatory marker C-reactive protein in early rheumatoid arthritis and proved to be of prognostic value in predicting clinical response to second-line therapy,<sup>35</sup> further supporting the above hypothesis. In the present study, plasma sCD30 levels were not statistically different in patients with chronic stable CAD, a well-known Th1-mediated disease,<sup>4</sup> compared to controls. However, *in vitro* sCD30 secretion was significantly lower in patients than controls, further complicating our understanding of its pleiotropic regulatory roles within the immune system. To our best of knowledge, the significance of sCD30 levels in patients with CAD has not been reported previously.

CD30 signaling has been demonstrated to prevent extensive expansion of autoreactive CD8<sup>+</sup> T cells upon secondary antigenic encounters in peripheral parenchymal tissues, such as pancreatic islets, thus protecting against autoimmunity.<sup>36</sup> Furthermore, antigen-induced CD4<sup>+</sup>CD25<sup>+</sup> regulatory T (Treg) cells have been found to suppress allograft rejection through enhanced memory CD8<sup>+</sup> T cell apoptosis, a finding which was largely dependent on the presence of CD30 on Treg cells and intact CD30-CD30L interaction. 37 Taken together, these studies illustrated a novel regulatory role for CD30 signaling in autoimmune diseases, i.e. CD30-mediated apoptosis of memory CD8<sup>+</sup> T cells, to eliminate self-reactive immune effector cells.<sup>12</sup> Current evidence supports an autoimmune mechanism in the pathogenesis of atherosclerosis, with oxidized low-density lipoproteins, heat shock proteins, and  $\beta 2$  glycoprotein I being identified as the culprit autoantigens in the development and progression of the disease.<sup>38,39</sup> Thus, decreased CD30<sup>+</sup> T cell populations and/or CD30 expression may potentially enhance atherogenesis through decreased elimination of autoreactive memory CD8<sup>+</sup> T cells. Nevertheless, the precise role of CD8<sup>+</sup> T cells in atherogenesis and plaque destabilization is yet to be elucidated.<sup>4</sup>

It is still unclear whether the progressive shift towards a Th1 immune response influences CD30 expression<sup>30</sup> or lower than normal CD30<sup>+</sup> T cell activity results in Th1 predominance.<sup>40</sup> In the latter case, manipulation of CD30-CD30L interaction will be of therapeutic value in Th1-mediated conditions. Furthermore, lower sCD30 levels in patients with angiographically confirmed CAD might represent an inherited susceptibility to the development of the disease, such as yet unidentified gene polymorphisms associated with autoimmunity.<sup>12</sup>

#### ACKNOWLEDGMENTS

This study was supported by grant from Tehran University of Medical Sciences and Health Services (88-04-30-9559).

#### REFERENCES

- Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005; 352(16):1685-95.
- Lundberg AM, Hansson GK. Innate immune signals in atherosclerosis. Clin Immunol 2010; 134(1):5-24.
- Andersson J, Libby P, Hansson GK. Adaptive immunity and atherosclerosis. Clin Immunol 2010; 134(1):33-46.
- Aukrust P, Otterdal K, Yndestad A, Sandberg WJ, Smith C, Ueland T, et al. The complex role of T-cell-based immunity in atherosclerosis. Curr Atheroscler Rep 2008; 10(3):236-43.
- Durkop H, Latza U, Hummel M, Eitelbach F, Seed B, Stein H. Molecular cloning and expression of a new member of the nerve growth factor receptor family that is characteristic for Hodgkin's disease. Cell 1992; 68(3):421-7.
- Schwab U, Stein H, Gerdes J, Lemke H, Kirchner H, Schaadt M, et al. Production of a monoclonal antibody specific for Hodgkin and Sternberg-Reed cells of Hodgkin's disease and a subset of normal lymphoid cells. Nature 1982; 299(5878):65-7.
- Tarkowski M. Expression and a role of CD30 in regulation of T-cell activity. Curr Opin Hematol 2003; 10(4):267-71.
- Del Prete G, De Carli M, Almerigogna F, Daniel CK, D'Elios MM, Zancuoghi G, et al. Preferential expression of CD30 by human CD4+ T cells producing Th2-type cytokines. FASEB J 1995; 9(1):81-6.
- Manetti R, Annunziato F, Biagiotti R, Giudizi MG, Piccinni MP, Giannarini L, et al. CD30 expression by CD8+ T cells producing type 2 helper cytokines. Evidence for large numbers of CD8+CD30+ T cell clones in human immunodeficiency virus infection. J Exp Med 1994; 180(6):2407-11.
- Hamann D, Hilkens CM, Grogan JL, Lens SM, Kapsenberg ML, Yazdanbakhsh M, et al. CD30 expression does not discriminate between human Th1and Th2-type T cells. J Immunol 1996; 156(4):1387-91.

- Bengtsson A, Johansson C, Linder MT, Hallden G, van der Ploeg I, Scheynius A. Not only Th2 cells but also Th1 and Th0 cells express CD30 after activation. J Leukoc Biol 1995; 58(6):683-9.
- Opat S, Gaston JS. CD30:CD30 ligand interactions in the immune response. Autoimmunity 2000; 33(1):45-60.
- Pellegrini P, Berghella AM, Contasta I, Adorno D. CD30 antigen: not a physiological marker for TH2 cells but an important costimulator molecule in the regulation of the balance between TH1/TH2 response. Transpl Immunol 2003; 12(1):49-61.
- 14. Pellegrini P, Totaro R, Contasta I, Berghella AM, Carolei A, Adorno D. CD30 antigen and multiple sclerosis: CD30, an important costimulatory molecule and marker of a regulatory subpopulation of dendritic cells, is involved in the maintenance of the physiological balance between TH1/TH2 immune responses and tolerance. The role of IFNbeta-1a in the treatment of multiple sclerosis. Neuroimmunomodulation 2005; 12(4):220-34.
- Contasta I, Berghella AM, Pellegrini P, Adorno D. Passage from normal mucosa to adenoma and colon cancer: alteration of normal sCD30 mechanisms regulating TH1/TH2 cell functions. Cancer Biother Radiopharm 2003; 18(4):549-57.
- 16. Krampera M, Vinante F, Tavecchia L, Morosato L, Chilosi M, Romagnani S, et al. Progressive polarization towards a T helper/cytotoxic type-1 cytokine pattern during age-dependent maturation of the immune response inversely correlates with CD30 cell expression and serum concentration. Clin Exp Immunol 1999; 117(2):291-7.
- Vinante F, Krampera M, Morosato L, Rigo A, Romagnani S, Pizzolo G. Peripheral T lymphocyte cytokine profile (IFNgamma, IL-2, IL-4) and CD30 expression/release during measles infection. Haematologica 1999; 84(8):683-9.
- Romagnani S, Del Prete G, Maggi E, Chilosi M, Caligaris-Cappio F, Pizzolo G, CD30 and type 2 T helper (Th2) responses. J Leukoc Biol 1995; 57(5):726-30.
- Horie R, Watanabe T. CD30: expression and function in health and disease. Semin Immunol 1998; 10(6):457-70.
- D'Elios MM, Romagnani P, Scaletti C, Annunziato F, Manghetti M, Mavilia C, et al. In vivo CD30 expression in human diseases with predominant activation of Th2like T cells. J Leukoc Biol 1997; 61(5):539-44.
- 21. Giacomelli R, Passacantando A, Parzanese I, Vernia P, Klidara N, Cucinelli F, et al. Serum levels of soluble CD30 are increased in ulcerative colitis (UC) but not in Crohn's disease (CD). Clin Exp Immunol 1998; 111(3):532-5.

- Munk ME, Kern P, Kaufmann SH. Human CD30+ cells are induced by Mycobacterium tuberculosis and present in tuberculosis lesions. Int Immunol 1997; 9(5):713-20.
- 23. Wang G, Hansen H, Tatsis E, Csernok E, Lemke H, Gross WL. High plasma levels of the soluble form of CD30 activation molecule reflect disease activity in patients with Wegener's granulomatosis. Am J Med 1997; 102(6):517-23.
- 24. Okumura M, Hidaka Y, Kuroda S, Takeoka K, Tada H, Amino N. Increased serum concentration of soluble CD30 in patients with Graves' disease and Hashimoto's thyroiditis. J Clin Endocrinol Metab 1997; 82(6):1757-60.
- Wakelkamp IM, Gerding MN, Van Der Meer JW, Prummel MF, Wiersinga WM. Both Th1- and Th2derived cytokines in serum are elevated in Graves' ophthalmopathy. Clin Exp Immunol 2000; 121(3):453-7.
- 26. Krams SM, Cao S, Hayashi M, Villanueva JC, Martinez OM. Elevations in IFN-gamma, IL-5, and IL-10 in patients with the autoimmune disease primary biliary cirrhosis: association with autoantibodies and soluble CD30. Clin Immunol Immunopathol 1996; 80(3 Pt 1):311-20.
- 27. Ichikawa Y, Yoshida M, Yamada C, Horiki T, Hoshina Y, Uchiyama M. Circulating soluble CD30 levels in primary Sjogren's syndrome, SLE and rheumatoid arthritis. Clin Exp Rheumatol 1998; 16(6):759-60.
- Gerli R, Caligaris-Cappio F, Bistoni O, Bertero MT, Giacomelli R, Falini B. Soluble CD30 in primary Sjogren's syndrome. Clin Exp Rheumatol 1999; 17(3):389-90.
- 29. Gerli R, Muscat C, Bistoni O, Falini B, Tomassini C, Agea E, et al. High levels of the soluble form of CD30 molecule in rheumatoid arthritis (RA) are expression of CD30+ T cell involvement in the inflamed joints. Clin Exp Immunol 1995; 102(3):547-50.
- Gerli R, Pitzalis C, Bistoni O, Falini B, Costantini V, Russano A, et al. CD30+ T cells in rheumatoid synovitis: mechanisms of recruitment and functional role. J Immunol 2000; 164(8):4399-407.
- Gerli R, Lunardi C, Vinante F, Bistoni O, Pizzolo G, Pitzalis C. Role of CD30+ T cells in rheumatoid arthritis: a counter-regulatory paradigm for Th1-driven diseases. Trends Immunol 2001; 22(2):72-7.
- 32. van Roon JA, van Roy JL, Duits A, Lafeber FP, Bijlsma JW. Proinflammatory cytokine production and cartilage damage due to rheumatoid synovial T helper-1 activation is inhibited by interleukin-4. Ann Rheum Dis 1995; 54(10):836-40.

IRANIAN JOURNAL OF ALLERGY, ASTHMA AND IMMUNOLOGY /241

- Walmsley M, Katsikis PD, Abney E, Parry S, Williams RO, Maini RN, et al. Interleukin-10 inhibition of the progression of established collagen-induced arthritis. Arthritis Rheum 1996; 39(3):495-503.
- 34. Joosten LA, Lubberts E, Durez P, Helsen MM, Jacobs MJ, Goldman M, et al. Role of interleukin-4 and interleukin-10 in murine collagen-induced arthritis. protective effect of interleukin-4 and interleukin-10 treatment on cartilage destruction. Arthritis Rheum 1997; 40(2):249-60.
- 35. Gerli R, Bistoni O, Lunardi C, Giacomelli R, Tomassini C, Biagini P, et al. Soluble CD30 in early rheumatoid arthritis as a predictor of good response to second-line therapy. Rheumatology (Oxford) 1999; 38(12):1282-4.
- 36. Kurts C, Carbone FR, Krummel MF, Koch KM, Miller JF, Heath WR. Signalling through CD30 protects against autoimmune diabetes mediated by CD8 T cells. Nature 1999; 398(6725):341-4.

- Dai Z, Li Q, Wang Y, Gao G, Diggs LS, Tellides G, et al. CD4+CD25+ regulatory T cells suppress allograft rejection mediated by memory CD8+ T cells via a CD30dependent mechanism. J Clin Invest 2004; 113(2):310-7.
- Mandal K, Jahangiri M, Xu Q. Autoimmune mechanisms of atherosclerosis. Handb Exp Pharmacol 2005; (170):723-43.
- Matsuura E, Kobayashi K, Matsunami Y, Shen L, Quan N, Makarova M, et al. Autoimmunity, infectious immunity, and atherosclerosis. J Clin Immunol 2009; 29(6):714-21.
- 40. Del Prete G, De Carli M, D'Elios MM, Daniel KC, Almerigogna F, Alderson M, et al. CD30-mediated signaling promotes the development of human T helper type 2-like T cells. J Exp Med 1995; 182(6):1655-61.

242/ IRANIAN JOURNAL OF ALLERGY, ASTHMA AND IMMUNOLOGY